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Use of AQUI-S®20E to Sedate Steelhead Trout and Sablefish in Saltwater

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Sedatives are chemicals or physical agents that—with increasing treatment concentration and duration—calm an animal and cause successive loss of mobility, equilibrium, consciousness, and reflex action. Fisheries professionals routinely sedate fish for a variety of purposes, including collection of samples or morphometric data, implantation of tags or tracking devices, and transport. Sedating fish before handling can minimize stress and physical injury to the fish and also help protect the handler. Ideally, a fish sedative is safe, effective, easy to administer, and inexpensive. Also, it is desirable that the sedative have no withdrawal period so that treated fish can be released into the wild immediately after treatment.

Currently, only tricaine methanesulfonate (tricaine or MS222) is approved by the U.S. Food and Drug Administration (FDA) for the temporary immobilization of fish and other aquatic, cold-blooded animals. The only tricaine product available in the U.S. is TRICAINE-S (Western Chemical, Inc., Ferndale, Washington USA). TRICAINE-S is effective and widely used by fisheries professionals; however, a 21-day withdrawal period is required for fishes entering the human food chain through stocking or slaughter and the product is not approved for marine fish species. For many field applications, holding fish for 21 days post-sedation is not practical and may seriously compromise management or research activities. Those working with marine fish species in a saltwater environment have fewer other suitable options.

In the U.S., efforts are underway to obtain FDA approval of AQUI-S®20E (10% eugenol; AQUI-S New Zealand, Ltd., Lower Hutt, New Zealand) as an immediate-release fish sedative. Considerable research has shown that eugenol is efficacious for sedating freshwater and marine fishes to handleable (e.g., Trushenski et al. 2012a, 2012b, 2012c). However, FDA requires data to demonstrate a product is effective in its final formulation at its lowest proposed efficacious dose. Effectiveness and safety data have been generated by the U.S. Fish and Wildlife Service (USFWS) to support approval of AQUI-S®20E for use to sedate all freshwater finfish to handleable. Data are now needed to support a similar claim for use on marine fish in a saltwater environment. As such, we conducted two independent trials to evaluate the efficacy of AQUI-S®20E for sedating juvenile Sablefish (*Anoplopoma fimbria*) and steelhead (anadromous *Oncorhynchus mykiss*) to the handleable stage.

Methods

Trials were conducted at the NOAA Manchester Research Station (Port Orchard, WA) in November 2015. Steelhead Trout were sedated to handleable with 250 mg/L AQUI-S®20E (25 mg/L eugenol) or 80 mg/L tricaine (active control). We tested 25 mg/L eugenol because it is likely the lowest efficacious dose that might be used on marine salmonids reared or held in cold water (i.e., <12.5°C) and was the dose used in trials to sedate freshwater salmonids (Bowker et al. 2014). Sablefish were sedated to handleable with 600 mg/L AQUI-S®20E (60 mg/L eugenol) or 180 mg/L Tricaine-S® (180 mg/L MS222). We tested 60 mg/L eugenol because it was the dose that consistently resulted in fish becoming handleable in 1 – 2 min and will likely be the lowest efficacious dose that might be used on bottom-dwelling marine fish reared or held in cold water (i.e., <12.5°C). A fish was determined to be handleable when it lost equilibrium and the ability to swim, could easily be caught by and held in hand, and did not struggle while being weighed or measured.

Sixty fish were used in each trial, whereby 30 fish were individually sedated under static conditions with each of the two sedatives. Working volumes of sedative solutions were prepared in bulk (60-gal) and used to fill individual sedation containers (1.5 gal in 5-gal plastic buckets for steelhead; 1.5 gal in 4.5 gal tubs for Sablefish). Contents of each sedation container were discarded after one fish had become sedated and removed from it. When a fish became handleable, it was removed from the sedative solution, measured for length and weight, and then transferred to a recovery vessel of static water. Static recovery baths were exchanged periodically so that dissolved oxygen (DO) concentrations never fell below 8 mg/L. Fish were considered recovered when they regained equilibrium, resumed normal swimming behavior, and could avoid obstacles placed in their path. Times to sedation and recovery were determined for each fish, and general fish behavior was assessed qualitatively during sedation and recovery. Following recovery, fish were returned to a holding tank supplied with fresh, flowing water and monitored for survival for 24 h.

Water temperature and dissolved oxygen (DO) concentration were measured in each sedation container before placing fish in

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the solution. Salinity and pH were measured once in untreated source water. In each trial, 20 sedative solution samples were randomly collected and analyzed to verify doses of eugenol by spectrophotometry.

Results and Discussion

All steelhead became handleable within 3.2 min (1.7 ± 0.6 min, mean ± SD) and recovered from sedation within 6.8 min (4.0 ± 1.3 min; Table 1). All Sablefish became handleable within 2.2 min (1.6 ± 0.2 min) and recovered from sedation within 12.4 min (7.3 ± 2.0 min; Table 1). Abnormal fish behavior (e.g., agitation, head-shaking, piping) was routinely observed among both species during exposure to AQUI-S®20E and MS222 (Table 2). There was no post-sedation mortality, and fish behavior was largely normal during recovery of both species (Table 2). Mean water temperature and DO concentration was 11.1°C and 9.0 mg/L for Sablefish and 12.5°C and 8.4 mg/L for Steelhead Trout (Table 3). Salinity and pH were within ranges suitable for rearing healthy cold water marine finfish (Table 3). Mean analytically verified doses of eugenol were within ±2% of target doses (Table 3).

Based on results from these trials, 250 mg/L AQUI-S®20E (25 mg/L eugenol) and 600 mg/L AQUI-S®20E (60 mg/L eugenol) were shown to effectively sedate steelhead and Sablefish, respectively, to handleable under the conditions tested. Although there are many factors (e.g., sedative concentration, fish size and life-stage, water temperature) that might influence time to sedation and recovery, we speculate that similar marine finfish (e.g., other marine salmonids or bottom-dwelling cold water fish) tested under similar conditions would become sedated and recover in comparable time periods. Results from these trials were submitted to FDA with a request to be included in the body of evidence supporting treatment efficacy of AQUI-S®20E.

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Table 1. Mean total length and weight (±1 SD), and mean (range) times to handleable and recovery for Sablefish and Steelhead Trout sedated to handleable with AQUI-S®20E or tricaine.

Species	Fish Size		Mean Time (min) to Sedation (range)		Mean Time (min) to Recovery (range)	
	Total Length (cm)	Weight (g)	AQUI-S®20E	Tricaine	AQUI-S®20E	Tricaine
Sablefish	26.6 ± 1.7	190.1 ± 32.8	1.6 (1.3 – 2.2)	1.1 (0.8 – 1.6)	7.9 (4.2 – 12.4)	7.0 (1.5 – 10.9)
Steelhead Trout	25.4 ± 3.8	166.0 ± 87.1	1.7 (1.0 – 3.2)	1.3 (0.9 – 2.1)	4.0 (2.5 – 6.8)	3.9 (1.7 – 8.1)

Table 2. Percentage of fish (out of 30) showing <u>abnormal</u> behavior (primarily slight agitation but also some instances of piping at the water surface and head-shaking) during sedative solution exposure and recovery from sedation.

	During Sedation		During Recovery	
Species	**AQUI-S®20E**	**Tricaine**	**AQUI-S®20E**	**Tricaine**
Sablefish	100%	93%	0%	0%
Steelhead Trout	67%	50%	3%	0%

Table 3. Water quality and eugenol dose verification results measured during the sedation trials.

	Water Quality Parameters (mean ±SD where reported)				Eugenol Concentration (mg/L)	
Species	**Temp (ºC)**	**DO (mg/L)**	**Salinity (ppt)**	**pH**	**Target**	**Actual (% from target)**
Sablefish	11.1 ± 0.1	9.0 ± 0.1	30.5	7.8	60	61.1 (+1.8%)
Steelhead Trout	12.5 ± 0.1	8.4 ± 0.2	30.4	7.5	25	25.1 (+0.3%)